Veterinary Pathology
47(3) 414-433

DOI: 10.1177/0300985810363902
http://vet.sagepub.com

A Morphological Study of 608 Cases of Canine Malignant Lymphoma in France With a Focus on Comparative Similarities Between Canine and Human Lymphoma Morphology

F. Ponce[1], T. Marchal[1], J. P. Magnol[1], V. Turinelli[1], D. Ledieu[1], C. Bonnefont[1], M. Pastor[1], M. L. Delignette[2], and C. Fournel-Fleury[1]

Abstract

This study reports cytomorphological, histomorphological, and immunological characterization of 608 biopsy cases of canine malignant lymphoma, with epidemiological and clinical data, collected from 7 French veterinary pathology laboratories. It compares morphological characteristics of malignant lymphoma in canines, per the updated Kiel classification system, with those reported in humans, per the World Health Organization (WHO) classification system. Of tumors described, 24.5% and 75.5% were classified as low- and high-grade malignant lymphomas, respectively. Presenting clinical signs included generalized or localized lymphadenopathy (82.4%) and extranodal diseases (17.6%) involving the skin (12.34%) and other sites (5.26%). Immunohistochemistry confirmed 63.8% B-cell (CD3–, CD79a+), 35.4% T-cell (CD3+, CD79a–), and 0.8% null-cell (CD3–, CD79a–) lymphomas. Most B-cell cases (38.49%) were of high-grade centroblastic polymorphic subtype; most T-cell cases (8.55%), high-grade pleomorphic mixed and large T-cell lymphoma subtypes. Some B-cell tumors showed morphologic characteristics consistent with follicular lymphomas and marginal zone lymphomas per the Revised European American Classification of Lymphoid Neoplasms and WHO canine classification systems and the WHO human classification system. Unusual high-grade B-cell subtypes included an atypical high-grade small B-cell lymphoma (0.66%), Burkitt-type B-cell lymphoma (1.64%), plasmacytoid lymphoma (0.99%), and mediastinal anaplastic large B-cell lymphoma (0.16%). Unusual T-cell subtypes included a previously undescribed high-grade canine immunoblastic T-cell type (1.15%), a rare low-grade prolymphocytic T-cell lymphoma (0.16%), and a recently described high-grade canine T-cell entity—aggressive granulocytic large-cell lymphoma (0.16%). Marginal zone lymphomas were common (10.86%); follicular lymphomas were rare (0.49%). Canine primary cutaneous malignant lymphoma subtypes were present (11.84%). There was no significant difference between B- and T-cell malignant lymphoma in regard to canine age and sex. A significant overrepresentation of Boxers (24.19%) was found for T-cell lymphomas.

Keywords

canine, classification, immunophenotyping, malignant lymphoma

Note: Article submitted 9th February 2004. Article accepted 22nd January 2010

Non-Hodgkin lymphomas (NHLs) are common tumors in dogs, with an incidence previously evaluated at 33 per 100,000.[49] They are also common in humans and are on noticeable increase.[1,5,7,46] As a close companion of humans that is subjected to the same environmental influences, the dog has often been proposed as a model for studying the etiology and therapy of NHLs. The value of this model depends on the possibility of distinguishing the main lymphoma subgroups in both species with a reliable classification of canine NHLs following the schemes currently used for human NHLs. The morphologic canine classifications[3,15,18,30,50,56] have been successively based on different human classifications—namely, the updated Kiel classification,[33] Revised European

[1] Laboratoire de Pathologie Clinique et Unité d'Oncologie, Ecole Nationale Vétérinaire de Lyon, France
[2] Unité de Biomathématiques, Ecole Nationale Vétérinaire de Lyon, France

Corresponding Author:
Frédérique Ponce, Unité de Médecine Interne, Ecole Nationale Vétérinaire de Lyon, 1 Avenue Bourgelat, 69280 Marcy L'Etoile, France
Email: f.ponce@vet-lyon.fr

American Classification of Lymphoid Neoplasms (REAL),[21,22] and more recently, the World Health Organization (WHO) classification[25,39,48]—taking account of epidemiological, clinical, phenotypic, and genetic features and identifying clinicomorphological entities.

The aims of this article are, first, to identify and describe the common B-cell, T-cell, and null-cell malignant lymphoma entities observed in canines in France using the updated Kiel classification system; second, to confirm previously obtained results concerning the high frequency of T-cell lymphomas in dogs as compared with their frequency in humans, using a large and statistically significant number of cases; and, third, to identify new, rare, or poorly described lymphoma subtypes in this large number of canine cases that earlier veterinary studies might have missed. Finally, we attempt a morphological comparison of disease entities of canine lymphoma, as defined by the updated Kiel classification system and the human WHO classifications, to view initial similarities and differences between canine and human lymphoma subtypes. This study presents a framework for further investigations, which will include developing new immunological markers and collecting additional epidemiological, clinical, immunological, and genetic data with a view to confirming the value of the dog species as a model for human NHLs.

Materials and Methods

Collection of Cases

This study was performed over 1 year, from May 2000 to May 2001. Histological paraffin-embedded specimens and case information for 702 biopsy admissions of canine lymphomas were collected from 7 private and university veterinary pathology laboratories in France. Laboratory selection was based on the number of pathologic cases diagnosed per year by each laboratory and on their geographic location in France. Fine-needle aspirates (FNAs) were available for all 93 cases submitted to the Laboratoire de Pathologie Clinique, Prof Fournel-Fleury.

From the initial 702 cases, 608 were included in the study. Ninety-four cases were excluded because of insufficient and inadequate tissue (n, 87) or misdiagnosis, including myeloid proliferation (n, 2) and benign lymphoid hyperplasia (n, 5), as confirmed by morphological and immunological tests and clinical follow-up. For all cases included in this study, the following data were collected: contributing laboratory, tumor topography, main clinical signs upon presentation, age, breed, and sex (without concern for sexual sterility of dogs). Tumor topography was assigned to one of the following categories: single lymph node involvement, generalized lymph node involvement, and extranodal involvement.

Histological Evaluation

Biopsy specimens from enlarged lymph nodes, extranodal masses, or cutaneous lesions in all 608 cases were fixed in 10% neutral buffered formalin at room temperature for 48 hours and embedded in paraffin wax by each laboratory. Tissue sections 5 μm in thickness were stained with hematoxylin and eosin (HE) and examined by one pathologist from each contributor laboratory to confirm a diagnosis of malignant lymphoma. Paraffin wax–embedded tumor tissues were subsequently sent to Laboratoire de Pathologie Clinique et Unité d'Oncologie Ecole Nationale Vétérinaire de Lyon, France, and sections were processed as described in previous studies.[4,15,16,18,40,42] Two pathologists (T.M. and associate) and one cytologist (C.F.F.) separately examined HE-stained slides before immunophenotyping. Finally, all authors examined all slides.

Cytological Evaluation

Cytological smears obtained by FNA of 93 nodal or extranodal masses were air-dried, fixed, and stained using the May–Grünwald–Giemsa technique as previously described.[4,15,16,18,40,42,54] Cytological slides were examined by one cytologist (C.F.F.). Morphological criteria based on the updated Kiel histological and cytological classification was the same as previously published.[15,16,18,40-42]

Immunophenotyping

Immunophenotyping was performed on paraffin wax–embedded sections from all 608 samples, with antibodies directed against human antigens but cross-reacting with the equivalent canine antigens (see Table 1). A polyclonal rabbit antibody against CD3 was used as a pan–T marker,[13] and a monoclonal antibody directed against CD79a was used as a pan–B marker (Dako, Glostrup, Denmark).[27,28] A standard avidin–biotin–immunoperoxidase procedure was implemented as described previously.[14,15,18] Immunophenotyping using pan–B markers and pan–T markers was examined by another scientist (F.P.) independently from the morphological study. Additional immunolabelings (Table 1) were performed only if initial immunophenotyping was insufficient to provide accurate tumor classification (51 cases). The same procedure was used as for paraffin-waxed sections.

Fourteen cases were evaluated for immunoglobulin characterization with antibodies to human kappa and lambda immunoglobulin light chain antigens that cross-reacted with equivalent canine antigens. These immunoglobulin antibodies were used in 9 potentially immunosecretory cases (because of their plasmacytic morphologic appearance) and in 5 null-cell lymphomas (CD3–, CD79a–) for additional investigation.[43,45] Canine-specific polyclonal antibodies detecting immunoglobulin G, immunoglobulin M (IgM), and immunoglobulin A were used in the same 14 cases.[15] The Ki-67 proliferation index was determined for only intermediate, atypical, or doubtful cases (n, 33), based on MIB-1 immunolabeling as previously described.[12,15,18]

Table 1. Antibodies Used for Immunocytohistochemical Staining of Canine Lymphomas

Primary Antibody	Dilution of Primary Antibody	Incubation Conditions	Origin (commercial reference)	First Description of Cross-Reactivity With Dog
Polyclonal antihuman pan–T cells (CD3)	1:50	1 h at room temperature	Dako	Ferrer et al[14]
Polyclonal anti-human kappa light chain	1:2000	30 min at 4°C	Dako	Sanduski et al[45]
Polyclonal anti-human lambda light chain	1:2000	30 min at 4°C	Dako	Sanduski et al[45]
Polyclonal anti-dog immunoglobulin G, Fc specific	1:2000	30 min at 4°C	Jackson Immunoresearch	
Polyclonal anti-dog immunoglobulin M, μ specific	1:2000	30 min at 4°C	The Binding Site	
Polyclonal anti-dog immunoglobulin A, Fc specific	1:2000	30 min at 4°C	Nordic Immunological Laboratories	
Monoclonal anti-human pan–B cells (CD79a)	1:25	1 h at room temperature	Dako (HM57)	Jones et al[28]
Monoclonal anti-human proliferating cells (Ki-67)	1:50	1 h at room temperature	Dako (MIB-1)	Falini et al[12]

Classification

Classification of the 608 cases was initially based on cellular morphology and immunophenotype according to the updated Kiel classification adapted to account for specific canine subtypes, as previously described.[15,18,40,42] B-cell morphologies that did not fit into the Kiel classification system—particularly, marginal zone lymphomas—were described according to the REAL and WHO human classifications and previous veterinary publications[15,18,40,42] and confirmed more recently by the REAL and WHO canine classification systems.[56]

Grading was determined by the size of cells (majority of small-sized cells or majority of medium- and large-sized cells) and by mitotic index (MI). Cases showing a majority of small-sized cells and a low or medium MI were classified as low-grade lymphomas, whereas those showing a majority of medium- and large-sized cells and a high MI were classified as high-grade lymphomas. For intermediate, atypical, and/or doubtful cases, final tumor subclassification was based on the MIB-1 immunolabeling results for Ki-67 index.[16]

Cutaneous T-cell lymphomas were identified as primary if there were tumor cell infiltration into the skin without evidence of visceral organ involvement. Secondary skin metastases from a primary visceral growth were not considered cutaneous tumors.

Canine Versus Human Morphologic Comparison

After clear tumor identification with the updated Kiel classification, a comparison was attempted for tumor histomorphology as reported in the human WHO classification system. B-cell morphologies that did not fit into the Kiel classification system, including marginal zone and follicular lymphomas, were described per the canine WHO classification system and then compared directly with the human WHO classification system.[48]

Statistical Analysis

Means were compared with Student t test, and variances were compared with the F test. Frequencies and distributions were compared with the Pearson chi-square test. Significance was taken as $P < .05$.

Results

Table 2 presents clinical and epidemiological data. Tables 3, 4, and 5 summarize morphological data. Tumor subtypes indicated in bold font in the tables represent new, recently recognized, and/or rare lymphoma subtypes as presented in canines in this study. Table 6 lists the respective proportion of cases of the various updated Kiel classification tumor subtypes in the canine species with morphological correlations with the human WHO classification subtypes.

General Findings, Epidemiology, and Clinical Data

There was no significant difference in age or sex distribution for B- and T-cell malignant lymphomas in canines (Table 2). Boxers appeared to be overrepresented (10.03%) among affected dogs, in comparison with an overall 2% of Boxers in the French canine population ($P < .0001$). The proportion of Boxers with T-cell lymphomas (24.19%) was significantly higher than that reported in the national canine population ($P < .0001$), whereas proportions of Boxers with B-cell lymphoma (2.32%) were not.

For all included dogs with malignant lymphoma, clinical signs of nodal involvement, either generalized or localized lymphadenopathy, were observed in 82.40% of cases. Extranodal disease (17.60% of cases) manifested most commonly as skin involvement (12.34%), whereas other sites (5.26%) included the digestive tract (1.48%), spleen (1.80%), tonsils (1.48%), and eyes (0.33%).

Table 2. Epidemiological and Clinical Features of Canine Lymphomas According to the Morphological Subtypes

			Sex		Lymphadenopathy		
Malignant Lymphoma Subtypes	No. Cases	Age in Years (Median)	Male	Female	Localized	Generalized	Extranodal Involvement
B-cell neoplasms	388	8.42	211	177	11	357	20
Small lymphocytic	2	10.50	1	1	0	2	0
Prolymphocytic	1	5.00	1	0	1	0	0
Lymphoplasmacytic	9	7.50	5	4	3	3	3
Centroblasto-centrocytic	3	10.25	2	1	0	3	0
Marginal zone	66	8.41	41	25	3	56	7
Small cells, not otherwise specified	4	7.00	2	2	0	4	0
Centroblastic monomorphic	5	7.25	3	2	0	3	2
Centroblastic polymorphic	234	7.81	117	117	0	232	2
Immunoblastic	47	9.05	28	19	3	43	1
Anaplastic/mediastinal B cell	1	6.00	1	0	0	0	1
Burkitt type	10	11.50	5	5	1	6	3
Plasmacytoid	6	10.10	5	1	0	5	1
T-cell neoplasms	215	8.22	123	92	10	118	87 + 1[a]
Small clear cell (T-zone)	20	9.17	9	11	9	10	1
Prolymphocytic	1	8.00	0	1	0	1	0
Pleomorphic small cell	5	8.38	4	1	0	3	2
Pleomorphic mixed	39	9.30	19	20	0	34	5
Pleomorphic large cell	13	6.67	9	4	0	13	0
Immunoblastic	7	6.84	3	4	0	7	0
Plasmacytoid	19	6.00	10	9	0	16	3
Large granular cell	1	5.00	1	0	0	1	1[a]
Lymphoblastic	17	6.85	11	6	0	17	0
Cutaneous	72	10.13	43	29	0	0	72
Unclassified	21	7.19	14	7	1	16	4
Null-cell neoplasms	5	8.40	1	4	0	5	0

[a] Extranodal involvement with lymphadenopathy.

Immunohistochemistry

Immunohistochemically, 388 cases (63.4%) were identified as CD3–, CD79a+ (B-cell lymphoma), and 215 cases (35.4%) were identified as CD3+, CD79a– (T-cell lymphoma). Five cases (0.8%) were negative for CD3 and CD79a and were therefore classified as null-cell lymphomas. Additional testing was performed in 51 cases that required further immunological data for accurate classification purposes, including 37 cases for Ki-67 expression and 14 cases of immunoglobulin characterization. Of these latter 14 cases, 9 with a plasmacytoid appearance suggesting possible immunosecretion (lymphoplasmacytic lymphomas) were positive for lambda immunoglobulin light chains and for IgM, whereas 5 were negative for all the immunolabelings, confirming their null-cell type.

Histomorphology and Cytomorphology

Tumor subtypes are described in the tables. Tumor subtypes that are unusual, poorly recognized, or rare (as identified in the tables in bold font) are described in greater detail. Remaining tumor subtypes have been well described within the literature.

B-cell Malignant Lymphoma. All 388 cases of B-cell malignant lymphoma (63.8%) were determined to represent mature B-cell lymphomas (Table 3). No precursor B-cell lymphomas (ie, lymphoblastic lymphoma) were observed. Mature B-cell lymphomas, mimicking normal stages of B-cell differentiation, have been classified and tabulated on the basis of their cell size, MI, and normal postulated counterpart.

High-grade B-cell malignant lymphomas were most common, with 234 cases of centroblastic polymorphic B-cell lymphoma, 5 centroblastic monomorphic cases (with 4 diffuse and 1 follicular subtype), 47 immunoblastic cases, 1 anaplastic mediastinal case, 10 Burkitt-type cases, 6 plasmacytoid cases, and 4 unusual cases of small-cell lymphoma not otherwise specified (NOS).

The single isolated new case of anaplastic mediastinal large B-cell subtype was identified by FNA on pleural effusion from a 6-year-old male German Shepherd with a mediastinal mass. Cytological examination of the pleural fluid showed large or giant cells, frequently binucleated or multinucleated and highly nucleolated, with irregularly clumped chromatin and extended highly basophilic cytoplasm (Fig. 1). Histologically, the mediastinal mass showed marked fibrosis and diffuse infiltration by large cells with pleomorphic nuclei, presence of binucleated cells, and extended highly basophilic cytoplasm (Fig. 2).

The 10 cases of Burkitt-type lymphoma showed a marked starry-sky pattern and diffuse infiltration by medium-sized cells with round nuclei, markedly clumped chromatin, several

Table 3. Morphology of the Various Subtypes of Canine B-Cell Lymphomas: 608 Cases[15]

Malignant Lymphomas	Cases No.	Cases %	Architectural Pattern	Cell Criteria	Mitotic Index (Ki-67)	Hyperplasia of PCV[a]	Starry Sky
Low-grade B cells	81	13					
Small lymphocytic aspect	2	<1	Diffuse	Small cells round nuclei, clumped chromatin, scant cytoplasm	2 (5%)	0 of 2	0 of 2
Prolymphocytic aspect[b]	1	<1	Diffuse	Small- to medium-sized cells round nuclei, condensed chromatin, central nucleolus relatively abundant cytoplasm	3	0 of 1	0 of 2
Lymphoplasmacytic aspect	9	1	Nodular	Small cells round nuclei, extended basophilic cytoplasm, plasma cell differentiation, expression of cytoplasmic lambda and cytoplasmic immunoglobulin M	3	0 of 9	0 of 9
Marginal zone	66	11	Diffuse or marginal zone	MMCs,[c] mixture of some immunoblasts and centroblasts, rarely irregular or monocytoid cells	3	0 of 66	0 of 66
Centroblastic/centrocytic	3	<1	Follicular	Centroblasts, centrocytes, large cleaved cells, FDC[d]	2	0 of 3	0 of 3
High-grade B cells	307	50					
Centroblastic monomorphic	4	<1	Diffuse	Medium- to large-sized cells round nuclei, fine chromatin, small nucleoli near the nuclear membrane, extended basophilic cytoplasm	7.7	0 of 5	4 of 5
	1		Follicular				
Centroblastic polymorphic	234	38	Diffuse	Mixture of MMCs (< 20%), small blastic cells (up to 80%), centroblasts (20 to 50%), immunoblasts (< 20%)	10	0 of 234	234 of 234
Immunoblastic	47	8	Diffuse	Mixture of immunoblasts (> 80%), MMCs (< 10%), centroblasts (< 10%), small blastic cells (< 10%)	9	0 of 47	47 of 47
Anaplastic/mediastinal	1	<1	Diffuse, fibrosis	Very large-sized cells, pleomorphic nuclei, binucleated cells, extended highly basophilic cytoplasm	16	0 of 1	0 of 1
Burkitt type	10	2	Diffuse	Medium-sized cells, round nuclei with highly clumped chromatin, multiple nucleoli, scant, deeply basophilic cytoplasm	24 (80%)	0 of 10	10 of 10
Plasmacytoid	6	1	Diffuse	Small- to medium-sized round nuclei; small nucleoli; fine to condensed chromatin; eccentric, extended, basophilic cytoplasm with pale juxtanuclear area	7	0 of 6	0 of 6
Small cells NOS[e]	4	1	Diffuse or nodular	Small- to medium-sized cells irregular nuclei, clumped chromatin, inconspicuous nucleoli	8.6 (65%)	0 of 4	0 of 4
Total	388	63.8				0	296

[a] PCV, postcapillary venules.
[b] Bolded subtypes indicate those which are unusual, rare, or previously unreported.
[c] MMCs, macronucleolated medium-sized cells.
[d] FDC, follicular dendritic cells.
[e] NOS, not otherwise specified.

Table 4. Morphology of the Various Subtypes of Canine T-Cell Lymphomas: 608 Cases[15,18,40,42]

Malignant Lymphomas	Cases No.	Cases %	Histological Pattern	Cytological Criteria	Mitotic Index (Ki-67)	Hyperplasia of PCV[a]	Starry Sky
Precursor T-cell lymphoma: Lymphoblastic	17	3	Diffuse	Medium-sized cells; round or convoluted nuclei with dusty chromatin, inconspicuous nucleoli, small ring of cytoplasm	10	3 of 17	5 of 17
Mature T-cell lymphoma	126	21					
Low-grade malignancy	26	4					
Small clear cell / T-zone	20	3	T-zone pattern	Interfollicular growth with follicle retention (T-zone pattern); small cells; round or slightly irregular nuclei; extended, unipolar, pale cytoplasm ("hand mirror" morphology)	1.3 (5.5%)	20 of 20	0 of 20
Prolymphocytic[b]	1	<1	Diffuse	Small cells; round nuclei, prominent nucleolus, clumped chromatin, small rim of cytoplasm	2	0 of 1	0 of 1
Pleomorphic small cell	5	<1	Diffuse	Small cells; indented nuclei, indistinct nucleolis, variably clumped chromatin, scant pale cytoplasm	1	0 of 5	0 of 5
High-grade malignancy	100	16					
Pleomorphic mixed[c]	39	6	Diffuse	Small-, medium-, and large-sized cells; irregular nuclei, pale cytoplasm, polymorphous background	9.3	0 of 39	0 of 39
Pleomorphic large cell[c]	13	2	Diffuse	Large-sized cells; irregular nuclei, pale cytoplasm	13	8 of 13	0 of 13
Immunoblastic	7	1	Diffuse	Majority of large-sized cells, markedly irregular nuclei; one or several voluminous nucleoli, extended pale cytoplasm	18	7 of 7	0 of 7
Plasmacytoid	19	3	Diffuse	Small-, medium-, and (less commonly) large-sized cells; eccentric round nuclei, visible nucleoli, irregularly clumped chromatin; abundant highly basophilic cytoplasm; pale juxtanuclear area; occasional binucleated cells; Giemsa positive	10	19 of 19	19 of 19
Aggressive large granular cell	1	<1	Diffuse	Small- to large-sized cells; hyperbasophilic cytoplasm, pale juxtanuclear area, coarse azurophilic granules	17 (83%)	0 of 1	0 of 1
Unclassified	21	3	Diffuse	Various aspects	12	0 of 21	0 of 21
Total	143	23.50				57	24

[a] PCV, postcapillary venules.
[b] Bolded subtypes indicate those which are unusual, rare, or previously unreported.
[c] Null-cell tumor morphology subtypes.

Table 5. Morphology of the Various Subtypes of Canine Cutaneous T-cell Lymphomas[15,18,40,42]

Malignant Lymphomas	No. Cases	Histological Pattern	Cytological Criteria	Mitotic Index
Low-grade malignancy	30			
Cerebriform small cell (ie, mycosis fungoides)	25	Epitheliotropic	Small cerebriform cells; irregular convoluted nuclei, single poorly visible nucleolus; epitheliotropism (epithelial/follicular), Pautrier microabscesses	2.2
Monocytoid	12	Dermal and Hypodermal	Small cells, common epitheliotropism (66.7% of cases); round nuclei, visible nuclear membrane, highly visible nucleolus; abundant pale cytoplasm, fine chromatin	3.5
Large granular lymphocytic	5	Dermal	Small- to medium-sized cells; rare epitheliotropism (20%); irregular nuclei, small nucleoli; extended pale cytoplasm with visible azurophilic granules; eosinophil inflammatory infiltrate in all cases	2.5
High-grade malignancy	42			
Cerebreform small and large cell in transformation	16	Diffuse	Small cerebriform cells with large irregular cell component; irregular convoluted nuclei, visible nucleoli; increased mitotic rate	12.5
Immunoblastic	8	Dermal	Large cells; no epitheliotropism; irregular indented nuclei, single large highly visible nucleolus; extended pale cytoplasm	9
Blastic	6	Dermal	Large cells; no epitheliotropism; round nuclei, small nucleoli adjacent to nuclear membrane; extended pale cytoplasm	12.5
Total	72			

nucleoli, and a small ring of hyperbasophilic cytoplasm (Figs. 3, 4). All exhibited a high MI (median, 24 per high-powered field [HPF], 400×) and Ki-67 index (80%). Three cases presented a slight plasmacytoid differentiation of 10% of cells with extended and eccentric hyperbasophilic cytoplasm. Three other cases showed 5% of atypical cells with slightly irregular nuclei, more prominent nucleoli, and the highest MI. Clinically, 6 Burkitt-type cases presented with generalized lymphadenopathy and 1 case with localized lymphadenopathy. The other 3 Burkitt-type cases had extranodal involvement: 1 with splenomegaly, 1 with enlarged tonsils, and 1 with uveitis.

The 6 provisional plasmacytoid B-cell lymphomas appeared slightly different from the Burkitt-type owing to a marked plasmacytoid appearance and fine to condensed chromatin in small- to medium-sized cells with no starry-sky pattern and a lower MI (median, 7 per HPF). Clinically, all presented with generalized lymphadenopathy except one, which presented mainly with skin nodules.

Small B-cell lymphomas generally have low mitotic activity and are considered a low-grade phenotype. The 4 cases of small-cell lymphoma NOS had nodular to diffuse infiltration of a typical small cell with irregular nuclei, inconspicuous nucleoli, and clumped chromatin (Figs. 5, 6) but a high mitotic rate and Ki-67 index (65%), suggesting a high-grade lymphoma phenotype (Fig. 7). These tumors also contained increased numbers of intermixed medium-sized cells.

Low-grade B-cell lymphoma subtypes included 2 small lymphocytic subtypes, 1 prolymphocytic subtype, 9 lymphoplasmacytic subtypes, 66 marginal zone lymphomas, and 3 centroblastic/centrocytic lymphomas with follicular architecture. The small lymphocytic lymphoma showed a diffuse infiltration by small cells (which are slightly larger than normal lymphocytes), scant cytoplasm, clumped chromatin, usually a round nucleus and occasionally a small nucleolus, and an interspersed prolymphocytic cell component (small- to medium-sized cells with relatively abundant cytoplasm, clumped chromatin, central nucleolus). The MI was medium (median, 2 per HPF), and the median Ki-67 index was 5%. There was no bone marrow or blood involvement.

The sole case of prolymphocytic lymphoma had a diffuse architectural pattern with small- to medium-sized cells identical to the prolymphocytes described above. The 9 lymphoplasmacytic lymphomas had a pseudofollicular organization of small cells with round nuclei, abundant basophilic cytoplasm, and perinuclear clearing, consistent with plasmacytoid differentiation. Additional immunohistochemical testing of these tumors showed positive cytoplasmic IgM and lambda immunoexpression, confirming plasma cell differentiation.

The group of marginal zone lymphomas (66 cases) was recognized as previously described in the REAL and WHO canine classification systems, both on architectural and cytological criteria. In most cases, the infiltrate was diffuse with some pseudonodular areas (58 cases), whereas in others (8 cases), the areas were highly pseudonodular with a clear marginal zone pattern (Fig. 8). Tumors often consisted of small- to medium-sized cells with a large central nucleolus, a low MI, and CD79a immunohistochemical positivity confirming a B-cell origin.

The 66 canine marginal zone cell lymphomas were divided into 3 subtype groups according to cytological criteria. The first group consisted of 59 cases (56 nodal and 3 extranodal lymphomas affecting spleen, tonsils, and nictitating membrane) that presented with macronucleolated medium-sized cells (MMCs). MMCs were characterized by a particularly prominent nucleolus in small- to medium-sized cells, with fine chromatin and a moderate amount of weakly basophilic cytoplasm (Fig. 9), as previously described.[17,35] A few large cells suggestive of centroblasts and immunoblasts were present in 29 of these 59 cases. The second group consisted of 5 cases (1 nodal and 4

Table 6. Updated Kiel Classification[33] of the 608 Canine Lymphomas With Possible Correlation With the World Health Organization Human Classification[25, 48]

Updated Kiel Malignant Lymphoma Classification	No. Cases	% Cases	World Health Organization Malignant Lymphoma Classification
B-cell neoplasms	388	64	
Low-grade malignancy	81	13	Peripheral B-cell neoplasms
Small B-cell lymphoma	12		
Small lymphocytic	2	<1	B-cell chronic lymphocytic leukemia / small lymphocytic lymphoma? *OR* low-grade small B-cell lymphoma not otherwise specified?
Prolymphocytic	1	<1	
Lymphoplasmacytic	9	1	Lymphoplasmacytic lymphoma
Marginal zone	66	11	Nodal marginal zone lymphoma Extranodal marginal zone lymphoma Splenic marginal zone lymphoma
Centroblasto-centrocytic	3	<1	Follicular lymphoma grade I/II
High-grade malignancy	307	51	
Centroblastic monomorphic	5		
Follicular subtype	1	<1	Follicular lymphoma grade III
Diffuse subtype	4	<1	Diffuse large B-cell lymphoma
Centroblastic polymorphic	234	38	Diffuse large B-cell lymphoma
Immunoblastic	47	8	Diffuse large B-cell lymphoma
Anaplastic/mediastinal	1	<1	Mediastinal B-cell lymphoma (diffuse large B-cell lymphoma)
Burkitt type	10	2	Burkitt lymphoma
Plasmacytoid	6	1	No World Health Organization correlate
Small cell not otherwise specified	4	<1	Mantle cell lymphoma?
T-cell neoplasms	215	35	
Precursor T-cell lymphoma: Lymphoblastic	17	3	Precursor T-cell lymphoblastic lymphoma/leukemia
Mature T-cell lymphoma			Peripheral T-cell and natural killer–cell neoplasms
Low-grade malignancy	26	13	
Prolymphocytic	1	<1	T-cell chronic lymphocytic leukemia / prolymphocytic leukemia
Pleomorphic small cell	5	<1	Peripheral T-cell lymphomas, unspecified
Small clear cell (T-zone)	20	3	Peripheral T-cell lymphomas, unspecified
High-grade malignancy	100	22	
Pleomorphic mixed	39	6	Peripheral T-cell lymphomas, unspecified
Pleomorphic large cell	13	2	Peripheral T-cell lymphomas, unspecified
Immunoblastic	7	2	Peripheral T-cell lymphomas, unspecified
Plasmacytoid	19	3	Peripheral T-cell lymphomas, unspecified
Aggressive large granular	1	<1	Enteropathy-type T-cell lymphoma *OR* natural killer leukemia *OR* extranodal nasal type
Unclassified	21	3	Unclassifiable
Cutaneous lymphoma	72		
Cutaneous T cell, low grade	53	9	Mycosis fungoides / Sezary syndrome
Cutaneous T cell, high grade	19	3	Cutaneous T-cell lymphoma
Null-cell neoplasms	5	<1	Natural killer–cell leukemia

digestive) characterized by monocytoid cells with paler and more abundant cytoplasm, which were interspersed with transformed blasts (Fig. 10). The third group concerned 2 cases with nodal involvement that were characterized by small- to medium-sized cells with slightly dispersed chromatin, inconspicuous nucleoli, irregular nuclei, and abundant cytoplasm (Fig. 11). Follicular lymphomas were uncommon (0.66%) and were recognized on the basis of a predominantly follicular pattern with a mix of centrocytes, centroblasts, and follicular dendritic cells (Figs. 12, 13).[25,48]

T-Cell and Null-Cell Lymphomas. T-cell lymphoma was identified in 215 cases (34.5%), including systemic and cutaneous manifestations (Table 4). The classification of T-cell lymphomas was first based on the fundamental distinction between precursor and mature T cells (17 cases and 177 cases, respectively). Unclassifiable T-cell tumors were present in 21 cases. Among mature T-cell lymphomas, the 72 primary cutaneous lymphomas were separated from the others, as is done in humans for morphologic classification.[25,48]

Precursor T-cell lymphoma. Lymphoblastic lymphoma appeared similar to previously reported cases, with a diffuse infiltration of a homogeneous proliferation of medium-sized cells with a small ring of moderately basophilic cytoplasm, a round to slightly convoluted nucleus, finely dispersed dusty chromatin, inconspicuous nucleoli, and a high MI (median, 10 per HPF) (Fig. 14).[40] The 17 dogs affected ranged from 3 to 11 years

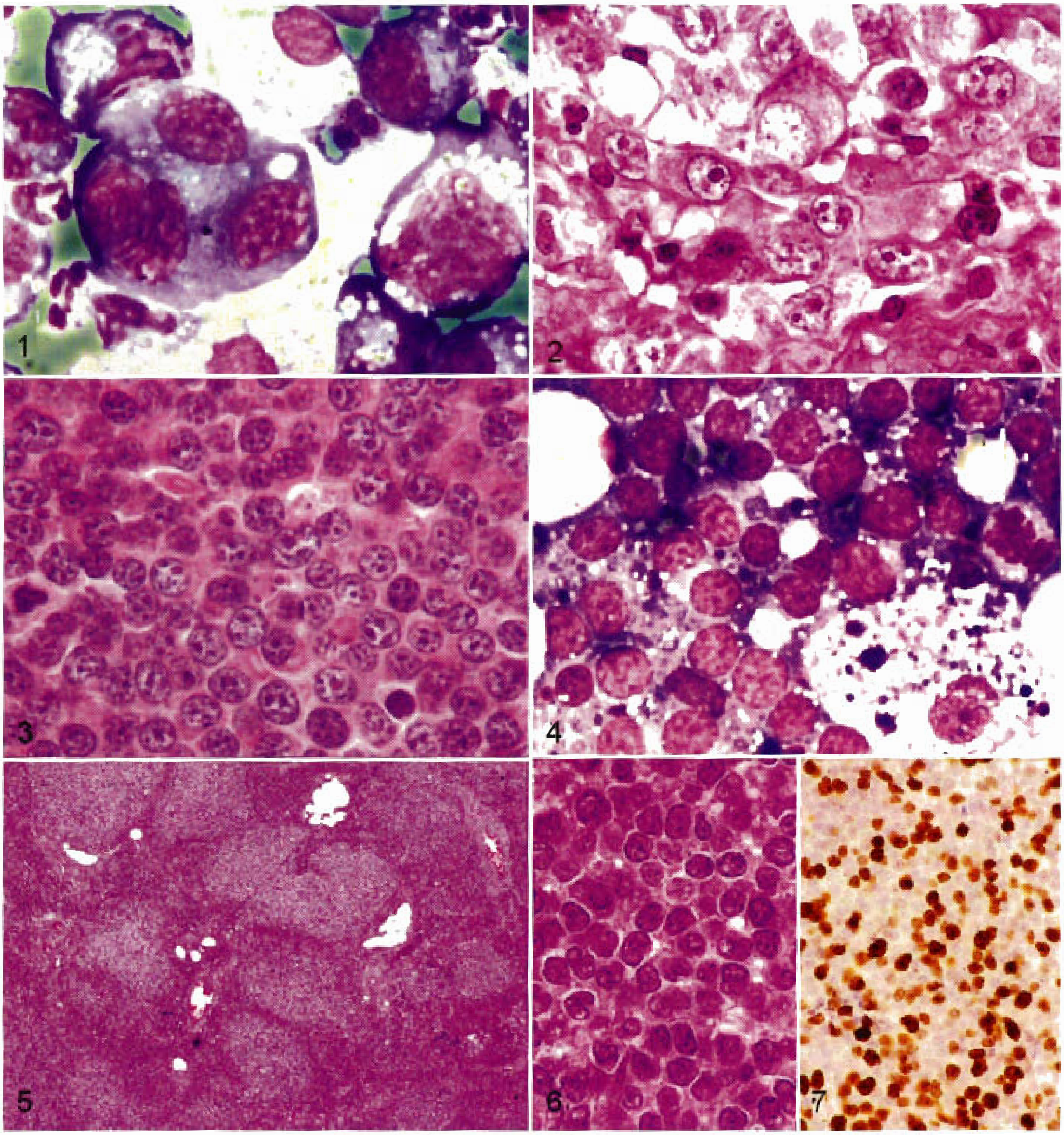

Figure 1. Pleural effusion. Fine-needle aspirate. Mediastinal B-cell lymphoma. Note the giant cells, with pleomorphic nuclei, irregularly clumped chromatin, and extensive basophilic cytoplasm. May-Grünwald-Giemsa technique. **Figure 2.** Mediastinal mass. Mediastinal B-cell lymphoma. Note the very large cells, with pleomorphic nuclei and extensive cytoplasm. HE. **Figure 3.** Lymph node. Burkitt-type B-cell lymphoma. Medium-sized cells with markedly clumped chromatin. HE. **Figure 4.** Lymph node. Fine-needle aspirate. Burkitt-type B-cell lymphoma. Medium-sized cells with round nucleus, markedly clumped chromatin, and several nucleoli. May-Grünwald-Giemsa technique. **Figure 5.** Lymph node. Small B-cell lymphoma, not otherwise specified. Diffuse to vague neoplastic nodular infiltration pattern. This pattern is similar to that described in previous cases of mantle cell lymphoma in dogs and humans. HE. **Figure 6.** Lymph node. Small B-cell lymphoma, not otherwise specified. Small cells with irregular nuclei, unconspicuous nucleoli, and clumped chromatin. HE. **Figure 7.** Lymph node. Small B-cell lymphoma, not otherwise specified. Note the very high proliferation index (65%). Ki-67.

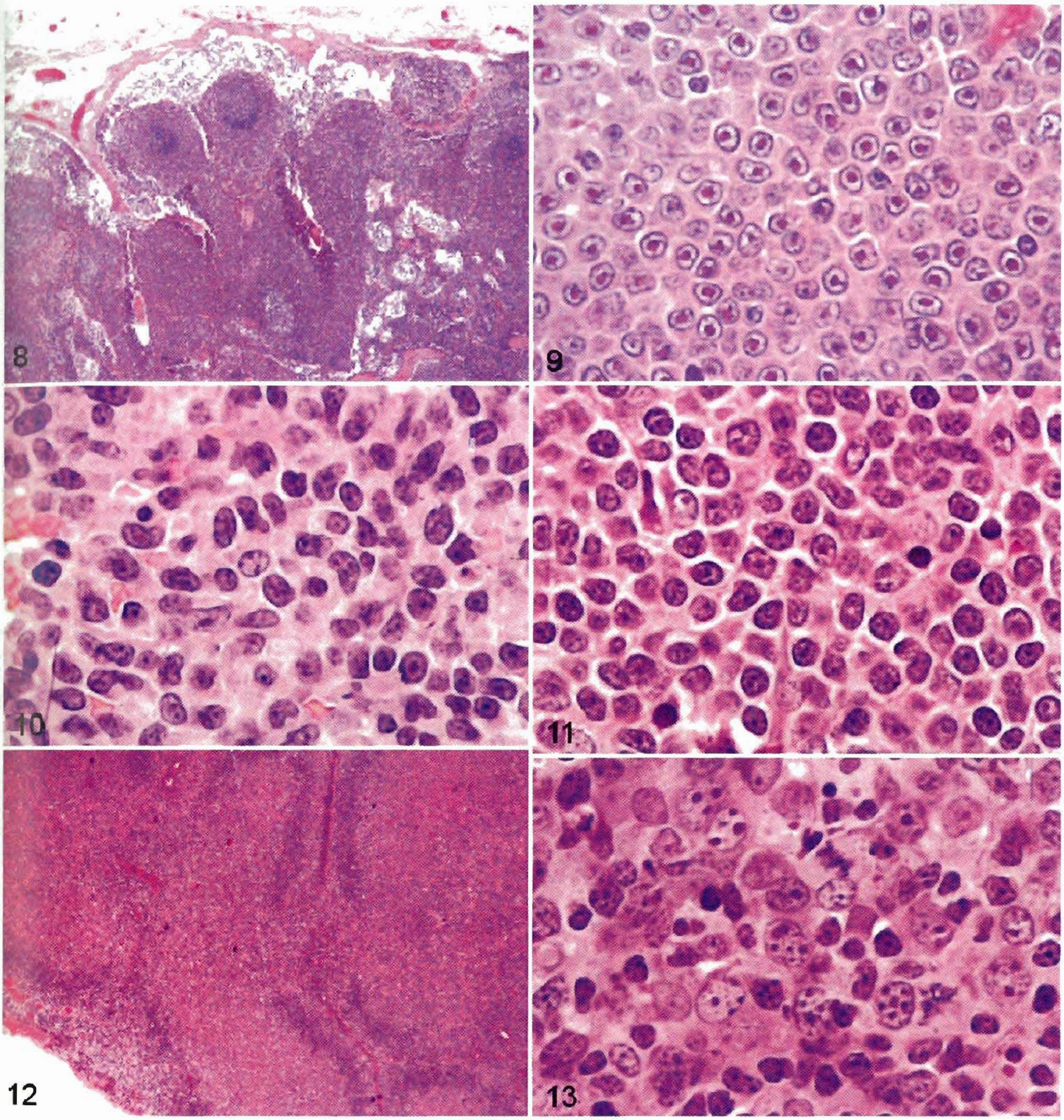

Figure 8. Lymph node. Marginal zone B-cell lymphoma. Characteristic marginal zone pattern. HE. **Figure 9.** Lymph node. Marginal zone B-cell lymphoma. Characteristic marginal zone cells (macronucleolated medium-sized cells), with a prominent nucleolus in small- to medium-sized nuclei, fine chromatin, and a moderate amount of cytoplasm. HE. **Figure 10.** Lymph node. Marginal zone B-cell lymphoma, histomorphologic variant. Monocytoid appearance (group 2). Note the pale and more abundant cytoplasm of the B cells when compared with those in Figure 9. HE. **Figure 11.** Lymph node. Marginal zone B-cell lymphoma, histomorphologic variant. Humanlike type (group 3). Small- to medium-sized cells, lightly dispersed chromatin, more or less visible nucleoli, slightly irregular nuclei, and abundant cytoplasm. HE. **Figure 12.** Lymph node. Follicular lymphoma. Characteristic follicular pattern. HE. **Figure 13.** Lymph node. Follicular lymphoma. Note the predominance of centroblast cells with some centrocytes. HE.

old (median age, 6.85 years), and all presented with polylymphadenopathy.

Mature or peripheral T-cell lymphomas. The majority of mature T-cell lymphomas identified in this study belonged to well-described subtypes as reported in previous studies.[15,18,42] High-grade T-cell lymphomas were most common with pleomorphic mixed-cell tumors present in 39 cases and pleomorphic large-cell tumors in 13 cases (Fig. 15). Remaining high-grade T-cell subtypes included 7 immunoblastic cases, 19 plasmacytoid cases,[40] and 1 recently described aggressive large granular lymphoma subtype.

A new, previously undescribed immunoblastic T-cell type occurred in 7 dogs ranging from 3 to 11 years old (median, 6.84 years). These 7 cases presented with generalized lymphadenopathy with diffuse nodal infiltration resulting in a moderate to pronounced starry-sky pattern. The lymphomatous population consisted of large cells with markedly irregular nuclei, fine chromatin, 1 voluminous nucleolus or several central nucleoli of variable size, and an extended pale cytoplasm (Fig. 16). The MI was high (median, 18 per HPF).

The plasmacytoid T-cell subtype was similar to that of previously reported cases,[42] with a population of small- to medium-sized cells or, less frequently, large cells and relatively abundant highly acidophilic cytoplasm. The nuclei were eccentrically located, round, or slightly notched (Fig. 17). This abundant, highly acidophilic cytoplasm—with eccentric nuclei, occasional binucleation of some cells, and juxtanuclear pale cytoplasmic area—provided a plasmacytoid appearance, which is obvious with Giemsa staining.

A rare subtype of aggressive granular large-cell lymphoma, only recently described in veterinary medicine,[8,19,53] was observed in 1 animal: A 5-year-old male Boxer presented with a primary intestinal mass, abdominal polylymphadenopathy, hepatosplenomegaly, and leukemia with 80% bone marrow infiltration. On tissue sections (Fig. 18), there was diffuse infiltration by cells of variable size with round or indented nuclei, fine or slightly clumped chromatin, inconspicuous or distinct nucleoli, and sometimes cytoplasmic azurophilic granules, without any angiocentric or angiodestructive growth pattern. Because samples of digestive tissue adjacent to the intestinal mass were not available, villous atrophy and/or enteropathy could not be evaluated.

On FNA examination (Fig. 19), the lymphomatous population was characterized by variable cell size with a majority of large cells, round and sometimes indented nuclei, fine or slightly clumped chromatin, inconspicuous or distinct nucleoli, and hyperbasophilic cytoplasm with a pale juxtanuclear area and coarse azurophilic granules. These neoplastic cells were intermingled with numerous reactive histiocytes displaying hemophagocytosis, and they had a high MI (median, 17 per HPF) and high Ki-67 proliferation index (83%). Low-grade T-cell lymphoma subtypes included 1 prolymphocytic case, 5 pleomorphic small-cell cases, and 20 cases of low-grade small clear cell–type (T-zone) lymphoma, as previously well described.[15,18]

The single case of prolymphocytic lymphoma had diffuse lymph node effacement by small cells with round or slightly irregular nuclei, a visible nucleolus, irregularly clumped chromatin, and a small rim of cytoplasm (Fig. 20). Leukemia was not observed in this case but was observed in the only 2 cases previously reported.[15]

All 5 cases of pleomorphic small-cell lymphoma had small cells with irregularly indented or deeply cleaved nuclei, and they exhibited the same morphological criteria as the 5 canine cases previously described.[15,18] The remaining 21 cases of T-cell lymphoma remained unclassifiable. All had an apparent high-grade morphology on the basis of their MI (median, 12 per HPF) but did not have enough characteristics to enter in a definite subtype.

Cutaneous T-cell lymphomas. Cutaneous tumors were all immunohistochemically consistent with T-cell lymphoma (72 cases) (Table 5). This study observed 6 provisional lymphoma subtypes. The 42 cases of low-grade cutaneous lymphoma included 25 cerebriform cell types with low MI (median, 2.2 per HPF), 12 monocytoid types, and 5 large granular lymphocytic types. The 30 cases of high-grade cutaneous lymphomas included 16 cerebriform cell types with high MI (median, 12.5 per HPF), 8 immunoblastic types, and 6 blastic types. The cerebriform cell type with low MI was consistent with the classically termed *mycosis fungoides* (MF).[15,18] However, the transitional to high-grade cerebriform subtype appeared to have some transformation with larger cells, visible nucleoli, and increased mitotic rate.

Monocytoid types comprised small cells with abundant pale cytoplasm, round nuclei containing fine chromatin, and a highly visible nucleolus, and they commonly showed epitheliotropism. Large granular lymphocytic types comprised medium-sized cells with moderate cytoplasm containing azurophilic granules, slightly irregular nuclei with clumped chromatin, and small nucleoli, and they rarely had evidence of epitheliotropism.

High-grade immunoblastic and blastic types were similar in the presence of large cells, with pale cytoplasm and an absence of epitheliotropism, but they differed in nuclear morphology, with immunoblastic cells having an irregular indented nucleus with a single large prominent nucleolus and with blastic cells having a round nucleus with multiple small nucleoli adjacent to the nuclear membrane.

Null-cell lymphomas. The 5 cases of null-cell lymphomas did not express CD3 and CD79a or light or heavy chains of immunoglobulin. Infiltration was diffuse; 4 cases were morphologically classified as pleomorphic mixed lymphomas and 1 as pleomorphic large-cell lymphoma. Descriptions are the same as those given for these high-grade T-cell lymphoma subtypes in Table 4.

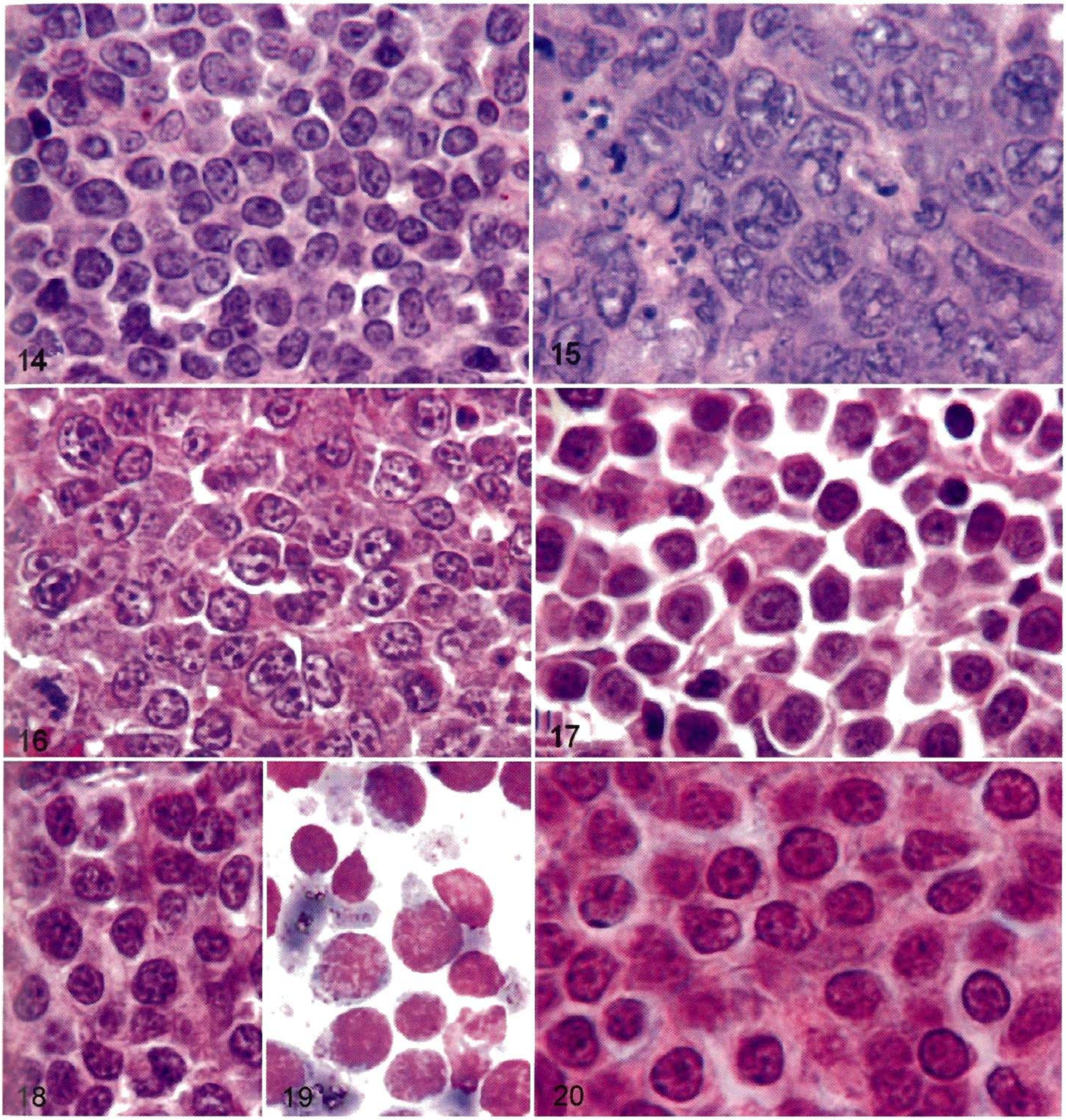

Figure 14. Lymph node. Lymphoblastic T-cell lymphoma. Homogeneous proliferation of medium-sized cells, with round nuclei, fine chromatin, and small but visible nucleoli. HE. **Figure 15.** Lymph node. Pleomorphic large T-cell lymphoma. Large cells with marked irregular nuclear outlines. HE. **Figure 16.** Lymph node. Immunoblastic T-cell lymphoma. Large cells with abundant, acidophilic, unipolar cytoplasm and a high nucleolar volume. HE. **Figure 17.** Lymph node. Plasmacytoid T-cell lymphoma. Medium- and large-sized cells, with extended, deeply acidophilic cytoplasm and pale juxtanuclear area in some cells. HE. **Figure 18.** Intestinal mass. Aggressive large granular T-cell lymphoma. Variable cell size, with round or indented nuclei, slightly clumped chromatin, and distinct nucleoli. HE. **Figure 19.** Lymph node. Fine-needle aspirate. Aggressive large granular T-cell lymphoma. Note the presence of coarse azurophilic granules in the cytoplasm. May-Grünwald-Giemsa technique. **Figure 20.** Lymph node. Prolymphocytic T-cell lymphoma. Majority of small cells with regular nucleiless commonly with slightly irregular nuclei, a visible nucleolus, irregularly clumped chromatin, and small rim of pale cytoplasm. HE.

Canine Versus Human Lymphoma: Morphologic Comparisons

B-cell Tumors. The single case of anaplastic mediastinal lymphoma in this case study was considered atypical because of the presence of fibrosis and binucleated neoplastic cells with pleomorphic nuclei and abundant cytoplasm; otherwise, it was consistent with that reported clinically and architecturally for the mediastinal variant of diffuse large B-cell lymphomas (DLBCLs) in humans (Table 6). In contrast to humans, this variant was observed in a middle-aged to older male dog, as opposed to the common human epidemiology of a younger female.[25,48]

Four canine cases were classified by the Kiel system as small-cell lymphoma NOS, which had characteristic small-cell morphology but a high mitotic rate and Ki-67 index, suggesting a high-grade lymphoma phenotype. The increased mitotic rate in conjunction with the small-cell morphology and the B-cell phenotype are suggestive of the reported human WHO classification of mantle cell lymphoma.[25,47,48,51] However, additional immunophenotyping to confirm this diagnosis was not performed, and further classification beyond the Kiel classification of small cell NOS was not possible.

Correspondence with human WHO classification for the low-grade canine B-cell Kiel classifications of 2 small lymphocytic cases and 1 small prolymphocytic case was difficult to establish because of an absence of certain morphological, immunophenotypic and genotypic features normally observed in humans. In humans, small lymphocytic lymphomas of the B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma subclass of the WHO classification are a neoplasm characterized by a pseudofollicular pattern with regularly distributed pale areas containing small lymphocytes admixed with prolymphocytes and paraimmunoblasts (larger cells with dispersed chromatin and more prominent central nucleolus). Owing to the lack of these architectural features and paraimmunoblasts and in the absence of more information on immunophenotype and cytogenetic abnormalities (ie, a scoring system that helps to differentiate chronic lymphocytic leukemia/small lymphocytic lymphoma from other B-cell entities in humans), these 2 canine small lymphocytic lymphomas were classified as unspecified low-grade small B-cell lymphomas.

Regarding the sole prolymphocytic case, the lack of blood and/or bone marrow involvement is consistent with the 2 canine cases previously published.[15] Despite the same morphological aspects as human B-cell prolymphocytic leukemia, the lack of leukemia in dogs could be more suggestive of a variant of small lymphocytic lymphoma with an increased number of prolymphocytes.[25,48]

Cases of canine low-grade B-cell lymphoplasmacytic lymphomas had cellular morphology and immunological findings that were suggestive of the human WHO classification of lymphoplasmacytic lymphoma, except for the growth pattern, which is diffuse for this tumor subtype in humans[21,25,48] rather than pseudofollicular as in canines. Based on this nodular orientation, plasmacytic differentiation of the cells appears more consistent with a human variant of marginal zone lymphoma with follicular colonization and plasma cell differentiation[43] than with the human lymphoplasmacytic lymphoma, which displays a diffuse pattern in the lymph node.

Marginal zone lymphomas in human medicine describe proliferation of morphologically heterogeneous small B cells, including marginal zone (centrocyte like) cells, monocytoid cells, small lymphocytes, and scattered transformed B cells[25,48,51] arranged as an encircling cuff that arises outside the mantle cell layer surrounding germinal centers. In humans, marginal zone lymphomas are separated into 3 subtypes: the nodal lymphoma and 2 subtypes that arise from extranodal sites, including MALT (mucosa-associated lymphoid tissue) lymphoma and splenic lymphoma. Canines in this study appeared to have similar subtypes as described above, with the last 2 canine subtype groups consistent with those described in cases of human marginal zone lymphoma.[25,48,51] The canine nodal version of marginal zone lymphoma, with the characteristic canine MMCs as previously described,[17,35] appeared most frequently (56 of 66). The extranodal sites in affected canines had a distribution similar to that reported in humans, with 6 apparent MALT sites affected (4 enteric, 1 tonsil, and 1 nictitating membrane) and 1 apparent splenic site affected. Whereas the majority of cases had characteristic canine MMCs, 5 cases (1 nodal, 4 digestive) showed a monocytoid appearance, and 2 other cases with nodal involvement showed a centrocyte-like appearance more consistent with the cell morphology described in cases of human marginal zone lymphoma.[21,23,25,48]

The 3 low-grade B-cell centroblasto-centrocytic lymphomas in this case study displayed a clear follicular pattern with a mix of centrocytes, centroblasts, and follicular dendritic cells as observed in human follicular lymphomas.[25,48] Their grading, as described in the human WHO classification,[25,48] is based on the proportion of large centroblasts to smaller centrocytes (grade 1 = 0–5 centroblasts per HPF, grade 2 = 6–15 centroblasts per HPF, grade 3 = 15+ centroblasts per HPF). In this study, the 3 canine cases could be classified as grade 2. In addition, the sole case of high-grade lymphoma described as centroblastic monomorphic with a follicular pattern could be classified as grade 3 follicular lymphoma according to the abundance of centroblasts.

T-cell Tumors. As in humans, precursor T-cell lymphoma (ie, lymphoblastic lymphomas) in dogs is rare (2.00% and 2.82% of all NHLs, respectively) and primarily of T-cell origin (85 to 90% and 100% in our study, respectively).[25,31,40,48] As reported in people, male dogs in this study appeared more commonly affected than females (11 vs 6), and dogs affected by this subtype are younger than those for the other T-cell subtypes (6.82 vs 8.22 years).[40] Cytologically, precursor T-cell lymphomas in dogs and humans had a similar cellular morphology and a high mitotic rate, as previously described.[40]

Most of the mature T-cell lymphoma subtypes observed in the dogs of this study were similar to the human WHO classification of peripheral T-cell lymphoma unspecified, including the low-grade pleomorphic small-cell and high-grade pleomorphic mixed, pleomorphic large-cell, and immunoblastic T-cell subtypes.[25,48]

Canine low-grade, small, clear-cell (T-zone) lymphoma, a well-established entity in dogs,[15,18] could be compared with the human T-zone lymphoma of the Kiel classification[33]—particularly, the clear-cell-rich subtype—on the basis of its histological pattern, cytological characteristics, and a more indolent course than that of other T-cell peripheral lymphomas. In our study, CD4 immunolabeling was not performed, but both cases of canine T-zone lymphoma previously published[18] were CD4+, as reported in humans.[33] This human Kiel subtype is described in the human WHO classification as a T-zone variant of the peripheral T-cell lymphoma unspecified.[25,48]

Nineteen cases of canine high-grade plasmacytoid T-cell lymphomas were observed in our study. Lennert[33] described a plasmacytoid T-cell lymphoma in the human Kiel classification; however, this category in humans has become obsolete in the WHO classification because further examination with specific antibodies and in situ probes has resulted in regrouping of entities. Furthermore, this human subtype had an indolent course (Ki-67 index, 2%), whereas our 19 cases appeared aggressive, with an MI of 10 per HPF.[25,48] Even though canine plasmacytoid T-cell lymphomas appeared clearly different from Lennert's human plasmacytoid T-cell lymphomas, the name *plasmacytoid* was conserved owing to the specific cell morphology (abundant highly acidophilic cytoplasm, pale juxtanuclear area, and eccentric round nuclei).

The single case of low-grade prolymphocytic lymphoma had morphological characteristics similar to those reported in humans with T-cell chronic lymphocytic leukemia/prolymphocytic leukemia, but the absence of leukemia in this canine case makes comparisons questionable and does not allow a definite classification.[25,48]

Cutaneous T-Cell

Human cutaneous T-cell lymphomas occur more frequently than do cutaneous B-cell lymphomas, and among them, MF and its variants are the most frequent (50% of cases),[6,10,11,36,48] similar to the cases observed in canines in this study (56%). Canine cases of cutaneous lymphoma with epitheliotropism and cerebriform cells are similar to those described with MF and Sezary syndrome in the human WHO classification.[25,48] In humans, it is well established that MF may transform into large T-cell lymphoma defined by the presence of more than 25% blast cells.[44] This form is more aggressive, with organ involvement and loss of epitheliotropism.[10,25,46,58] In this study, there were 16 cases that probably represent transformed MF, characterized by a mixture of large blastic cells and small cells with cerebriform nuclei and by a high MI (median 12.5 per HPF) as suggested by previously published studies.[18]

Discussion

The predominance of high-grade lymphomas (75.49%) over low-grade lymphomas (24.51%), the high frequency of large B-cell lymphomas (47.38%) and pleomorphic T-cell lymphomas (9.37%), and the rarity of follicular lymphomas (0.66%) in dogs are consistent with the findings of previous veterinary studies.[15,18,20,50] From an epidemiological viewpoint, this study did not show any difference in the mean age (8 years) of dogs suffering from lymphomas when compared with previous veterinary studies.[20,26,49,50] The correlation between the age of dogs and morphological lymphoma subtypes (eg, lymphoblastic T-cell and plasmacytoid T-cell lymphomas in younger dogs and cutaneous lymphomas in older dogs)[18,40,42] and the overrepresentation of Boxers in the whole of lymphomas, particularly in T-cell lymphomas, are consistent with preestablished data.[18,26,34,36,38,40,42,50] No sex predilection was observed in this study of canine lymphomas, a highly debated point in previous studies.[18,26,29,49]

Regarding frequency of T-cell lymphoma in dogs in comparison with humans, examination of a statistically significant proportion of canine cases of lymphoma in France showed that 35.4% of affected animals have T-cell lymphoma, confirming findings reported in previous studies.[16,18,49] In this study, the proportion of canine T-cell lymphoma cases, which proved much higher than that in humans, was clearly related to the frequency of cutaneous T-cell lymphoma (33.49% of T-cell NHLs). The frequency of cutaneous T-cell lymphoma observed here appears much higher than that reflected by the percentages reported in previous veterinary morphological studies.[18,20,50] The increased number of cases observed in this study could be explained by the sampling of study materials from pathology laboratories, which are more likely to receive skin masses from veterinary practices (ie, masses that are easily observed and surgically removed). An increase in the number of skin samples compared with lymph node or other organ samples would result in an apparent increase in the proportion of cutaneous lymphoma cases.

Clinically, the low frequency of extranodal involvement in canine lymphomas (18%) in comparison with human lymphomas (30%)[24,25,46,48] should be cautiously interpreted because of the retrospective nature of this study and the primary use of histological materials. Available clinical data mainly consisted of predominating presenting signs and profound lesions, which could have underestimated the degree of extranodal involvement in favor of superficial involvement (eg, superficial lymphadenopathy and cutaneous lymphomas). Additional clinical data relating to the different canine lymphoma subtypes are necessary to determine the clinical profile of each entity.

This study highlights the proportion of cases and morphological aspects of common canine lymphoma subtypes, as well as those rarer subtypes that could have been underestimated in more restricted, previously published studies. In addition to morphologic descriptions, some apparent morphologic similarities were observed among canine malignant lymphoma subtypes (as identified with the Kiel classification system in this large population from 7 different veterinary laboratories throughout France) and among the human malignant lymphoma subtypes (as reported in the WHO classification).

Canine Lymphoma Morphology

B-cell Lymphomas. B-cell lymphoma subtypes, including the high-grade large B-cell lymphomas of the centroblastic polymorphic subtype and the immunoblastic subtype, were the most common in this canine population, and they have been well documented in previous veterinary studies.[15,20] However, unusual or rare B-cell lymphoma subtypes were observed, including high-grade types such as anaplastic mediastinal, Burkitt type, and small-cell lymphoma NOS, as well as low-grade marginal zone and follicular lymphomas. In addition, a provisional subtype of high-grade Burkitt-like lymphoma with plasmacytoid morphology was identified (discussed in the comparative section below).

In this study, canine Burkitt-like tumor morphology was similar to the 3 previously published cases in veterinary medicine.[4,15,56] All cases showed a high growth fraction, with 80% of cells positive for Ki-67. This specific morphology is an important subtype to be recognized by pathologists because of the propensity for a poor prognosis as previously published.[41]

Small B-cell lymphomas generally have low mitotic activity and are considered a low-grade phenotype. The 4 cases of small B-cell lymphoma NOS observed here had a classic small-cell morphology but a high mitotic rate, averaging 8.6 per HPF, compared with the usual small B-cell mitotic rate, ranging from 2 to 3 per HPF. The diffuse or nodular pattern observed in the 4 unusual cases in this study of canine small cell NOS, along with the small-cell morphology, the high MI, and the B-cell immunophenotype, may be compared to that of the 3 canine cases of mantle cell lymphoma described in a recent study by Valli et al.[57] They described 3 cases of canine splenic mantle cell lymphoma on the basis of histological and cytological criteria—namely, proliferation of small cells with internal nuclear detail, without nucleoli, and centered on end arterioles. However, in this study, the lack of cyclin D1 positivity[9,25,48] and absence of monoclonal antibodies specific to or cross-reacting with canine CD5 did not allow definite identification of a mantle cell subtype.

Among the low-grade lymphomas, the typical canine nodal marginal zone cell subtype (marginal zone lymphoma) is well defined in the literature and is clearly recognizable because of its marginal zone pattern of growth and characteristic cytological presentation, including specific MMCs. However, this study pointed out a rare cytological presentation not previously described in veterinary studies—including small- to medium-sized cells, slightly dispersed chromatin, inconspicuous nucleoli, irregular nuclei, and abundant cytoplasm—observed in 2 of the 66 canine nodal marginal zone lymphomas.

Regarding extranodal marginal zone lymphomas in this canine population, MALT and splenic marginal zone lymphomas appeared rare and slightly different from those of a recent canine study, which reported 8 tumors with exclusive splenic involvement among 46 marginal zone lymphomas.[57] Cases may be underestimated in our study given that digestive and splenic biopsies are not systematically performed in animals with evidence of lymphoma in the lymph node and/or skin.

Follicular lymphomas are well defined in the veterinary and human WHO classification systems but were not specifically described in the Kiel classification system. Follicular lymphomas in canines in this study were rarely observed, as in previous veterinary studies.[4,15,20,49,51,56,57]

In addition, there was an absence of lymphoblastic B-cell lymphoma, also known as precursor B-cell lymphoma, in this significant European series, which is consistent with previous reports in French canine populations.[15,40] This result based on the Kiel classification system is slightly different from a recent veterinary book that described some cases of lymphoblastic B-cell lymphoma.[56]

T-cell Lymphomas. The morphological presentation of most T-cell lymphomas is highly specific, hence enabling pathologists to strongly suspect T-cell phenotypes.[15,18] In regard to morphological presentation and proportion of cases, the findings relating to T-cell lymphomas in this study are similar to those reported in recent veterinary publications.[18,49,50,55] The low-grade pleomorphic small-cell lymphomas and high-grade pleomorphic mixed and large-cell lymphomas constituted the majority of nodal T-cell lymphomas identified.

Two previously provisionally classified tumors, considered specific T-cell subtypes on the basis of their clinical behavior, were observed in greater numbers than those previously reported.[41] These included the low-grade small clear-cell (T-zone) lymphoma and high-grade plasmacytoid lymphoma.[15,18,31,37,40,41,46,49,50] Most important, 7 cases of high-grade immunoblastic T-cell lymphoma were observed and so represent the first identified cases of an immunoblastic T-cell subtype in canines.

Two questionable types of canine T-cell lymphoma were named as provisional entities: one case of low-grade T-prolymphocytic lymphoma and one case of aggressive high-grade large granular cell lymphoma. The single case of T-prolymphocytic lymphoma appeared to be similar to the 2 previously published canine cases.[15] This may constitute a rare category of low-grade canine T-cell lymphoma/leukemia described in the updated canine Kiel classification, but further clinical evaluation is necessary for confirmation.

The single case of aggressive large granular lymphocytic T-cell lymphoma appeared to have a similar morphology to that of the 6 cases described in recent veterinary studies.[8,19,53] The low number of cases observed in these studies may be due in part to the sampling of study materials from tissue biopsies easily removed from superficial lesions in veterinary practice, whereas the clinical presentation of this type of lymphoma consists of profound infiltration (spleen, liver, abdominal lymph nodes) without superficial, visible lesions.

Finally, 21 unclassifiable T-cell lymphomas were defined as those that did not fit a definitive or provisional category of the updated Kiel classification system. Morphological, immunological, and clinical data were insufficient to venture an assumption concerning the cells of origin. Further immunophenotypic and genetic studies should be carried out on these tumors for classification purposes.

Cutaneous T-cell Lymphoma. In this study, cutaneous T-cell lymphomas occurred more frequently than B-cell lymphomas. Small-cell cerebriform epitheliotropic tumors (25 cases), which likely represent MF and its variants, were the most common (56%).[36,56] For a long time, canine cutaneous small-cell lymphomas were most commonly classified as MF based on the presence of epitheliotropism and CD3 positivity. However, 2 distinct morphologic subtypes have epitheliotropism and CD3 positivity but may be different from MF—namely, monocytoid-type lymphoma (12 cases in this study) and large granular lymphocytic lymphoma (5 cases).

Monocytoid-type lymphoma differs from MF by the presence of homogeneous cells with small round nuclei containing one highly visible nucleolus and abundant pale cytoplasm and by the absence of small cells with cerebriform nuclei as observed in cases of MF. We are unsure of the clinical course of this subtype of lymphoma and whether it exhibits the same clinical features as MF (patches, plaques, and nodules) before treatment. Prospective studies are absolutely necessary to point out clinical and therapeutic differences between monocytoid-type lymphoma and MF, if any.

Large granular lymphocytic lymphoma differs from MF primarily by the presence of cytoplasmic acidophilic granules but shows a more regular nucleus with more condensed chromatin and small nucleoli. Similar cases have shown a high proliferation rate with a poor response to chemotherapy and usually a TiA-1+ immunophenotype (personal observation).

Null-Cell Lymphoma. Publications on null-cell lymphomas are rare.[43,53] One recently reported a null-cell phenotype for aggressive large granular lymphocyte lymphomas, diagnosed owing to the presence of cytoplasmic granules.[53] In our study, the null-cell cases were classified as pleomorphic subtypes on the basis of cell morphology in the absence of cytoplasmic granules. TiA-1 or other cytotoxic markers were not available for further classification. Null-cell types remain immunologically poorly characterized because of the lack of canine CD56 immunomarkers to identify natural killer cell lymphomas, as tested by Turinelli et al.[53]

Canine Versus Human Lymphoma: Morphologic Comparison

B-cell Lymphoma. High-grade large B-cell lymphomas of the centroblastic polymorphic subtype and the immunoblastic subtype, which were the most common tumors observed in this study, appear morphologically similar to the DLBCLs described in the REAL and WHO human classifications.[24,25] These findings are well documented and consistent with previous veterinary and human studies.[13,15,20,24,25,46,49]

The only case of anaplastic mediastinal B-cell lymphoma observed during our study appears similar to that described in a recent veterinary study,[56] and it probably also falls within the category of DLBCL. Along with other high-grade B-cell neoplasms, including centroblastic polymorphic, diffuse centroblastic monomorphic, and immunoblastic B-cell lymphoma, mediastinal lymphomas are considered part of DLBCL as described in the human WHO classification system based on cell size, irregular nuclear outlines, abundant cytoplasm, and association with nodular sclerosis.[25,48]

As in dogs, this DLBCL group of B-cell tumors is reported to be the most common lymphoid neoplasm subtype in human adults, as well as almost all other species. In humans, DLBCL tumors consist of sheets of large transformed B lymphocytes that architecturally obliterate the normal tissue morphology and occur at nodal and extranodal sites—particularly, enteric and mucosal (MALT) sites—as was observed in dogs with these tumor subtypes. In canines but not humans (as yet), MMCs are commonly observed in centroblastic polymorphic and immunoblastic lymphoma cases, as intermixed with large blastic cells, suggesting transformation of these tumor types from marginal zone lymphoma.[41]

Human Burkitt-type lymphomas, classified as a high-grade B-cell NHL, are characterized by a more pronounced starry-sky pattern than are other fast-growing lymphomas, a high mitotic and apoptotic rate, medium-sized cells, round nuclei, clumped chromatin, and relatively clear parachromatin.[25,48] In this study, canine Burkitt-like tumor morphology was similar to that of the human subtype and the 3 previously published cases in veterinary medicine.[4,15] Moreover, a high growth fraction was observed in canine tumors, with 80% of cells positive for Ki-67, which is close to the almost 100% Ki-67 positivity reported in human Burkitt-type lymphoma.[13,25,48]

Diagnosis of the provisional highly aggressive plasmacytoid B-cell lymphoma raises the question of a possible variant of a Burkitt-type lymphoma (plasmacytoid variant) as it exists in the human WHO classification but with some morphological differences, including smaller cells, finer chromatin, and the absence of a single central nucleolus and a starry-sky pattern. Alternatively, consideration must be given to transformation of a generally low-grade small B-cell lymphoma toward a higher grade owing to the presence of small- to medium-sized cells with higher MI.[25,47,48]

The 4 canine cases of high-grade small B-cell lymphoma NOS present in this case study had morphologic similarities to the 3 canine cases of mantle cell lymphoma described in a recent study by Valli et al.[57] The mantle cell lymphoma is one of the worst malignant lymphomas in humans, with a poor median survival time (less than 3 to 5 years).[25,47,48] This high-grade small-cell lymphoma does not undergo transformation to a large-cell lymphoma, but a high MI (> 20 per HPF, 10×), peripheral blood involvement, and/or increase in nuclear size and chromatin dispersal are associated with a poor survival time. Morphologic variants are reported for human mantle cell lymphomas, such as blastoid, pleomorphic, marginal zone–like, and particularly small lymphocytic types. This last type may be difficult to differentiate from low-grade small B-cell lymphomas such as small lymphocytic lymphomas.[48] In humans, immunophenotyping (CD5 and cyclin D1 positive, CD10 and CD23 negative) and genotypic/cytogenetic features—translocation t(11;14)(q13;q32)—are usually sufficient to make this differential diagnosis.[9,25,47,48] Immunophenotyping for immunoglobulin heavy chain, cyclin D1, or gene translocations as reported in humans was not performed in either canine study owing to insufficient development of canine cytogenetic analyses.[57] In some cases, a definitive diagnosis among small B-cell lymphoma subtypes is difficult because of overlapping morphologic and immunophenotypic features and/or lack of specific genotypic anomalies. In these cases, only an early diagnosis with a preserved architecture allowing the observation of a particular mantle cell pattern, a precise morphological description, and the clinical outcome are useful to distinguish the highly aggressive mantle cell lymphomas from the other indolent small B-cell lymphomas.

The different Kiel subtypes of canine low-grade small B-cell lymphoma identified in this study, including small lymphocytic, prolymphocytic, and lymphoplasmacytic subtypes, were difficult to classify and compare with the various well-known entities of the human WHO classification. In the human WHO classification, the group of small B-cell lymphomas includes B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma; mantle cell lymphoma; splenic, nodal, or MALT marginal zone lymphomas; and lymphoplasmacytic and follicular lymphomas.[9,25,47,48]

Small B-cell tumors are generally indolent, but a variable proportion may undergo transformation to large-cell lymphomas.[47] The small size of the B cells is not enough to predict clinical behavior, so it is important for the pathologist to make a precise morphological analysis of each case and to perform a proliferation index, such as Ki-67, in addition to the classical MI to differentiate mantle cell lymphomas that, at least in humans, have a worse prognosis. Additional studies are important for these tumors in canines as well because they often require intensive evaluation, including early diagnosis and further diagnostics.

In regard to marginal zone lymphoma, the presence of specific MMCs has been described in canines[15,17,35] but appears to constitute an original difference from its human equivalent.[23-25,48] This study pointed out a rare cytological presentation described earlier in 2 of the 66 canine nodal marginal zone lymphomas, which had not been described in veterinary studies. However, the cytological morphology is close to that described for the classical form of human marginal zone cells.[48,51,52] In regard to extranodal marginal zone lymphomas, the specific cytological presentation of the infiltrates of monocytoid cells interspersed with transformed blasts in the 4 extranodal digestive mucosal-associated canine cases described in this study resembled their human equivalents.[23,48]

Follicular lymphomas are well defined in the veterinary and human WHO classification systems.[15,25,48,56,57] In this study, the 4 cases of follicular lymphoma could be divided into different morphological grades, as described in the human WHO classification.[25,48] Three cases showed a mixture of centrocytes and centroblasts (centroblastic–centrocytic type) and could be classified as grade 2. The fourth case, showing a majority of centroblasts (centroblastic monomorphic type) and high MI, could be classified as grade 3. Follicular lymphoma appears generally uncommon in dogs, but this trend is in opposition to reports in humans, which are more common.[25,48] Differences regarding incidence of this subtype among species may be explained either by a late diagnosis in dogs or, more likely, by the rapid and diffuse invasion of highly proliferating blastic forms of follicular origin.[15,20,49,51] For more questionable cases, the bcl-2 protein, expressed in the majority of human cases, should be further investigated in dogs.[25,48]

T-cell Lymphoma. The human WHO classification of unspecified peripheral T-cell lymphoma is a general catchall that is becoming obsolete in people because of the increased use of additional genetic and diagnostic tests. However, the absence of similar testing available for canine species results in the classification of most canine T-cell tumors identified by the Kiel classification system into this human WHO subtype—including low-grade pleomorphic small-cell lymphoma, low-grade small clear-cell (T-zone) lymphoma, high-grade pleomorphic mixed and large-cell lymphomas, high-grade immunoblastic T-cell lymphomas, and high-grade plasmacytoid lymphomas.

The single case of aggressive large granular lymphocytic T-cell lymphoma observed in this study was more difficult to classify. Aggressive to fulminant clinical course, multiorgan involvement, cytoplasmic azurophilic granules within neoplastic cells, and intermingled reactive histiocytes with hemophagocytic syndrome, as reported in this case, are close to clinical and morphological features described in human aggressive natural killer cell leukemia/lymphoma or extranodal natural killer cell/T-cell lymphoma, nasal type. Because of the presence of a primary intestinal mass, this case may also be compared with human enteropathy-associated T-cell lymphoma. However, characteristic epitheliotropism of neoplastic cells found in human cases of enteropathy-associated T-cell type, along with celiac disease, was not found.[24,25,37,48] Further investigations, mainly immunophenotypical (CD56, granzyme M) and genetic (presence of T-cell receptor gene rearrangement or not), should be carried out to distinguish which of the tumors from this larger group of canine aggressive large granularlymphocytic T-cell lymphoma arise from the innate immune system (aggressive natural killer cell leukemia/lym phoma or extranodal natural killer cell/T-cell lymphoma, nasal type) or from the adaptive immune system (enteropathy-associated T-cell lymphoma).[25,48]

The single case of provisionally named T-prolymphocytic lymphoma observed in this study, which is similar to the 2 previously published canine cases,[14] may constitute a rare updated Kiel classification category of low-grade canine T-cell lymphoma/leukemia. However, further clinical and immunophenotypical investigations (especially, presence of leukemia, aggressive clinical course, and *TCL1* oncogene overexpression) are required to compare it with the T-prolymphocytic lymphoma/leukemia of the human WHO classification.[25,48]

Finally, unclassifiable T-cell lymphomas were defined as those that did not fit either a definitive or provisional category of the updated Kiel classification system and therefore could not be further evaluated. Morphological, immunological, and clinical data were insufficient to venture an assumption concerning the cells of origin. Further immunophenotypic and genetic studies should be carried out on these tumors for classification purposes.

Cutaneous T-cell Lymphoma. In humans, primary cutaneous lymphomas may present a specifically skin-oriented morphology, such as MF, or resemble their nodal counterparts but with a different phenotype, genotype, or clinical behavior, such as cutaneous follicular lymphomas.[25,48] Canine cases of cutaneous lymphoma in this study with epitheliotropism and cerebriform cells are most consistent with MF and/or Sezary syndrome in the human WHO classification.[25,48] Several other primary cutaneous human lymphomas—including cutaneous gamma–delta T-cell lymphoma, primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma, or extranodal T-cell lymphoma of nasal type—share common histomorphological and immunohistochemical features with MF, such as epitheliotropism, a small pleomorphic cell component, and either surface or cytoplasmic CD3 positivity. Only aggressive or highly aggressive behavior and usual positivity for cytotoxic proteins (granzyme, perforin, TiA-1) distinguish them from MF.[32]

It is more difficult to classify the other 14 cases of cutaneous lymphoma, which are composed only of large blastic cells, including 8 immunoblastic and 6 blastic types. Among these high-grade lymphomas, some may result from the complete transformation of small-cell lymphomas (such as MF), but the others are probably real primary large-cell lymphomas, as in humans.[25,32] In humans, primary cutaneous large T-cell lymphomas expressing CD30 antigen bear a more favorable prognosis. Unfortunately, no canine CD30 antibodies were available, and human monoclonal antibodies usually used to recognize CD30 antigen do not cross-react with canine lymphocytes (personal observation).[2,6,32]

This large and significant population of dogs with malignant lymphoma has confirmed some previously published information as well as shown evidence of new cytohistological entities present in the French population. Moreover, many clinicomorphological entities initially characterized with the updated Kiel classification for canine NHLs appear to have similarities to those reported in the human WHO classification system, including Burkitt-type lymphoma, immunoblastic T-cell lymphoma, aggressive large granular T-cell lymphoma, various subtypes of cutaneous T-cell lymphoma, and others. To truly determine the significance of canine malignant lymphoma in relation to human malignant lymphoma, consistent classification of lymphoma subtypes between the 2 species would be useful. Although direct comparison between Kiel lymphoma subtypes and WHO classification are difficult because of the requirement of clinical data and cellular response to treatment in the Kiel classification system, this initial morphologic comparison between the 2 species will serve as a springboard for further studies.

Acknowledgements

We wish to thank the following 7 veterinary pathology laboratories for their valuable contribution to our study by providing the histological material: Laboratoire d'Histo-Cytopathologie Vétérinaire, Drs Mialot and Lagadic, Maisons-Alfort; Laboratoire de Pathologie Clinique, Ecole Vétérinaire de Lyon, Prof Fournel-Fleury, Prof Magnol; Laboratoire Vetopath, Drs Pitou, Breton, and Sales, Sophia Antipolis; Laboratoire d'Anatomie Pathologique Vétérinaire, Metz; Laboratoire d'Anatomie Pathologique Vétérinaire du Sud Ouest, Drs Poujade and Degorce, Toulouse; Laboratoire d'Anatomie Pathologique Vétérinaire, Drs George, Lenet, Longeart, and Amboise; Laboratoire d'Anatomie Pathologique, Ecole Vétérinaire de Nantes, Prof Wyers. We also wish to thank S. Balleydier and C. Bonnefont-Rebeix for their technical support and Dr Christy McKnight for her editorial input. This study was funded by the Association pour la Recherche contre le Cancer, Fondation pour la Recherche Médicale, Ligue contre le Cancer Région Rhône-Alpes, Fondation de France, Académie de Médecine, and Ecole Nationale Vétérinaire de Lyon.

Declaration of Conflicting Interests

The authors declared that they had no conflicts of interest with respect to their authorship or the publication of this article.

Financial Disclosure/Funding

This study was funded by the Association pour la Recherche contre le Cancer (ARC), Fondation pour la Recherche Médicale (FRM), Ligue contre le Cancer Région Rhône-Alpes, Fondation de France, Académie de Médecine, Ecole Nationale Vétérinaire de Lyon.

References

1. Anderson JR, Armitage JO, Weisenburger DD: Epidemiology of the non-Hodgkin's lymphomas: Distributions of the major subtypes differ by geographic location. Ann Oncol **9**:717–720, 1998.
2. Baines SJ, McCormick D, McInnes E, Dunn JK, Dobson JM, McConnel I: Cutaneous T cell lymphoma mimicking cutaneous histiocytosis: differentiation by flow cytometry. Vet Rec **147**:11–16, 2000.
3. Breuer W, Hermanns W: Classification of lymphohaemopoietic neoplasias (LHN) in dogs and cats. Eur J Vet Pathol, **4**:5–20, 1998.
4. Carter RF, Valli VE, Lumsden JH: The cytology, histology and prevalence of cell types in canine lymphoma classified according to the National Cancer Institute Working Formulation. Can J Vet Res **50**:154–164, 1986.
5. Cartwright R, Brincker H, Carli PM, Clayden D, Coebergh JW, Jack A, McNally R, Morgan G, De Sanjose S, Tumino R, Vornanen M: The rise in incidence of lymphomas in Europe (1985–1992). Eur J Cancer **35**:627–33, 1999.
6. Cerroni L, Gatter K, Kerl H. An Illustrated Guide to Skin Lymphoma, 2nd ed. Blackwell, Malden, MA, 2004.
7. Chassagne-Clément C, Blay JY, Treilleux I, Sebban C, Bergeron C, Biron P, Philip T: Epidemiology of non-Hodgkin's lymphoma: recent data. Bull Cancer **86**:529–536, 1999.
8. Cienava EA, Barnhart KF, Brown R, Mansell J, Dunstan R, Credille K: Morphologic, immunohistochemical, and molecular characterization of hepatosplenic T-cell lymphoma in a dog. Vet Clin Pathol **33**:105–110, 2004.

9. Coelho Siqueira SA, Ferreira Alves VA, Beitler B, Otta MM, Nascimento Saldiva PH. Contribution of immunochemistry to small B-cell lymphoma classification. Appl Immunohistochem Mol Morphol **14**:1–6, 2006.
10. Connors JM, Hsi ED, Foss FM: Lymphoma of the skin. Hematology **1**:263, 2002.
11. Day MJ: Immunophenotypic characterisation of cutaneous lymphoid neoplasia in the dog and cat. J Comp Pathol **112**:79–96, 1995.
12. Falini B, Flenghi L, Fagioli M, Stein H, Schwarting R, Riccardi C, Manocchio I, Pileri S, Pelicci PG, Lanfrancone L: Evolutionary conservation in various mammalian species of the human proliferation-associated epitope recognized by the Ki-67 monoclonal antibody. J Histochem Cytochem **37**:1471–1478, 1989.
13. Felman P, Gentilhomme O: Atlas de cytopathologie ganglionnaire. Arnette, Paris, France, 1997.
14. Ferrer L, Fondevila D, Rabanal R, Ramis A: Detection of T lymphocytes in canine tissue embedded in paraffin wax by means of antibody to CD3 antigen. J Comp Pathol **106**:311–314, 1992.
15. Fournel-Fleury C, Magnol JP, Bricaire P, Marchal T, Chabanne L, Delverdier A, Bryon PA, Felman P: Cytohistological and immunological classification of canine malignant lymphomas: comparison with human non-Hodgkin's lymphomas. J Comp Pathol **117**:35–39, 1997.
16. Fournel-Fleury C, Magnol JP, Chabanne L, Ghernati I, Marchal T, Bonnefont C, Bryon PA, Felman P: Growth fractions in canine non-Hodgkin's lymphomas as determined in situ by the expression of the Ki-67 antigen. J Comp Pathol **117**:61–72, 1997.
17. Fournel-Fleury C, Magnol JP, Marchal T, Chabanne L, Bryon PA, Felman P. An original perifollicular zone cell in the canine reactive lymph node: a morphological, phenotypical and aetiological study. J Comp Pathol **113**:217–231, 1995.
18. Fournel-Fleury C, Ponce F, Felman P, Blavier A, Bonnefont C, Chabanne L, Marchal T, Cadore JL, Goy-Thollot I, Ledieu D, Ghernati I, Magnol JP: Canine T-cell lymphomas: a morphological, immunological and clinical study of 46 new cases. Vet Pathol **39**:92–109, 2002.
19. Fry MM, Vernau W, Pesavento PA, Bromel C, Moore PF. Hepatosplenic lymphoma in a dog. Vet Pathol **40**:556–62, 2003.
20. Greenlee PG, Filippa DA, Quimby F, Patnaik AK, Calvano SE, Matus RE, Kimmel M, Hurvitz AI: Lymphomas in dogs: a morphologic, immunologic and clinical study. Cancer **66**:480–490, 1990.
21. Harris NL: Introduction and rationale for the REAL classification. *In*: Human Lymphoma: Clinical Implications of the REAL Classification, ed. Mason DY and Harris NL, pp. 1.1–1.7. Springer-Verlag, London, UK, 1999.
22. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JKC, Cleary ML, Delsol G, De Woolf-Peeters C, Falini B, Gatter KC, Grogan TM, Isaacson PG, Knowles DM, Mason DY, Mueller-Hermeling HK, Pileri SA, Piris MA, Ralfkiaer E, Warnke RA: A revised European-American classification of lymphoid neoplasms: a proposal from the international study group. Blood **84**:1361–1392, 1994.
23. Isaacson PG, Spencer J: Malignant lymphoma of mucosa associated lymphoid tissue. Histopathology **11**:445–462, 1987.
24. Jaffe ES: Morphologic, immunologic and genetic features of peripheral T cell lymphomas (unspecified category). *In*: Human Lymphoma: Clinical Implications of the REAL Classification, ed. Mason DY and Harris NL, pp. 27.1–27.8. Springer-Verlag, London, UK, 1999.
25. Jaffe ES, Harris NL, Stein H, Vardiman JW: World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon, France, 2001.
26. Jagielski D, Lechowski R, Hoffmann-Jagielska M, Winiarczyk S: A retrospective study of the incidence and prognostic factors of multicentric lymphoma in dogs (1998–2000). J Vet Med A Physiol Pathol Clin Med **49**:419–424, 2002.
27. Jones M: Peptide Immunization as a Source of Cross-Species Reactive Antisera Recognizing Leukocyte Differentiation Antigens. Immunological Reagents for the Study of Disease in Companion Animals. Wellcome Trust, London, UK, 1993.
28. Jones M, Cordell JL, Beyers AD,Tse AGD, Mason DY: Detection of T and B cells in many animal species using cross-reactive anti-peptide antibodies. J Immunol **150**:5429–5435, 1993.
29. Keller ET, Mc Ewen EG, Rosenthal RC, Helfand SC, Fox LE: Evaluation of pronostic factors and sequential combination chemotherapy with doxorubicin for canine lymphoma. J Vet Intern Med **7**:289–295, 1993.
30. Kiupel M, Teske E, Bostock D: Pronostic factors for treated canine malignant lymphoma. Vet Pathol **36**:292–300, 1999.
31. Knowles DM: Morphologic, immunologic and genetic features of precursor T and B cell neoplasms. In: Human Lymphoma: Clinical Implications of the REAL Classification, ed. Mason DY and Harris NL, pp. 43.1–43.2. Springer, London, UK, 1999.
32. Leboit PE, Burg G, Weedon D, Sarasin A, ed.: WHO Classification of Tumours: Pathology and Genetics of Skin Tumours. IARC Press, Lyon, France, 2006.
33. Lennert K, Feller CA: Histologie des Lymphomes Malins Non Hodgkiniens Selon la Classification de Kiel Actualisée. Doin, Paris, France, 1991.
34. Lurie DM, Lucroy MD, Griffey SM, Simonson E, Madewell BR. T-cell derived malignant lymphoma in the boxer breed. Vet Comp Oncol **2**:171, 2004.
35. Magnol JP, Fournel-Fleury C, Marchal T, Chabanne L, Bryon PA, Felman P: Lymphomes malins à moyennes cellules macronucléolées dans l'espèce canine: implication d'une cellule originale de la zone marginale du ganglion lymphatique réactionnel. Bull Acad Natl Med **1**:51–65, 1995.
36. Magnol JP, Ghernati I, Marchal T, Chabanne L, Delverdier A, Fournel-Fleury C: Données cliniques, morphologiques et immunophénotypiques à partir de 10 observations de lymphome T cutanéo-muqueux épithéliotrope du chien (analogue au mycosis fongoide) Intérêt d'un modèle animal de pathologie spontanée. Bull Acad Natl Med **180**:449–462, 1996.
37. Medeiros LJ: Intermediate and high-grade diffuse non-Hodgkin's lymphomas in the working formulation. *In*: Surgical Pathology of the Lymph Nodes and Related Organs, ed. Jaffe ES, 2nd ed., pp. 283–343. WB Saunders, Philadelphia, PA, 1995.

38. Modiano JF, Breen M, Burnett RC, Parker HG, Inusah S, Thomas R, Avery PR, Lindblad-Toh K, Ostrander EA, Cutter GC, Avery AC: Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk. Cancer Res **65**:5654–5661, 2005.
39. Pileri SA, Milani M, Fraternali-Orcioni G, Sabattini E: From the REAL to the upcoming WHO scheme: a step toward universal categorization of lymphoma entities? Ann Oncol **9**:607–612, 1998.
40. Ponce F, Magnol JP, Blavier A, Bonnefont C, Ghernati I, Felman P, Buff S, Fournel-Fleury C: Clinical, morphological and immunological study of 13 cases of canine lymphoblastic lymphoma: comparison with the human entity. Comp Clin Path **12**:75–83, 2003.
41. Ponce F, Magnol JP, Ledieu D, Marchal T, Turinelli V, Chalvet-Monfray K, Fleury-Fournel C: Canine malignant lymphoma morphological subtypes: an essential step for prognosis of dogs under chemotherapy. Vet J **167**:158–166, 2004.
42. Ponce F, Magnol JP, Marchal T, Chabanne L, Ledieu D, Bonnefont C, Felman P, Fournel-Fleury C: High-grade canine T-cell lymphoma/leukemia with plasmacytoid morphology: a clinical spathological study of nine cases. J Vet Diagn Invest **15**:330–7, 2003.
43. Ruslander DA, Gebhard DH, Tompkins MB, Grindem CB, Page RL. Immunophenotypic characterization of canine proliferative disorders. In Vivo **11**:169–172, 1997.
44. Salhany KE, Cousar JB, Greer JP, Casey TT, Fields JP, Collins RD: Transformation of cutaneous T-cell lymphoma to large cell lymphoma:. a clinicopathologic and immunologic study. Am J Pathol **132**:265–277, 1988.
45. Sandusky GE, Horton PJ, Wightman KA: Use of monoclonal antibodies to human lymphocytes to identify lymphocyte subsets in lymph nodes of the rhesus monkey and dog. J Med Primatol **15**:441–451, 1986.
46. Solal-Celigny P, Brousse N, Fermé C, Gisselbrecht C, Reyes F, Coiffier B: Lymphomes, Lymphomes non Hodgkiniens–Maladie de Hodgkin, 3rd ed. Aubin Press, Poitiers, France, 1997.
47. Swerdlow SH: Small B-cell lymphomas of the lymph nodes and spleen: practical insights to diagnosis and pathogenesis. Mod Pathol **12**:125–140, 1998.
48. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, ed.: World Health Organization Classification of Tumours. Haematopoietic and Lymphoid Tissue. IARC Press, Lyon, France, 2008.
49. Teske E: Canine malignant lymphoma: a review and comparison with human non-Hodgkin's lymphomas. Vet Q **4**:209–19, 1994.
50. Teske E, Wisman P, Moore PF, Van Heerde P: Histologic classification and immuno-phenotyping of canine non-Hodgkin's lymphomas: unexpected high frequency of T cell lymphomas with B cell morphology. Exp Hematol **22**:1179–1187, 1994.
51. Thieblemont C, Nasser V, Felman P, Leroy K, Gazzo S, Callet-Bauchu E, Loriod B, Granjeaud S, Gaulard P, Haioun C, Traverse-Glehen A, Baseggio L, Bertucci F, Birnbaum D, Magrangeas F, Minvielle S, Avet-Loiseau H, Salles G, Coiffier B, Berger F, Houlgatte R: Small lymphocytic lymphoma, marginal zone B-cell lymphoma, and mantle cell lymphoma exhibit distinct geneexpression profiles allowing molecular diagnosis. Blood **103**:2727–37, 2004.
52. Traverse-Glehen A, Felman P, Callet-Bauchu E, Gazzo S, Baseggio L, Bryon PA, Thieblemont C, Coiffier B, Salles G, Berger F: A clinicopathological study of nodal marginal zone B-cell lymphoma: a report on 21 cases. Histopathology **48**:162–73, 2006.
53. Turinelli V, Marchal T, Ponce F, Bonnefont-Rebeix C, Fournel-Fleury C: Aggressive large granular lymphocytes lymphomas associated with a hemophagocytic syndrome in 5 dogs: a clinical cytohistological and immunological study. Comp Clin Path **13**:109–118, 2005.
54. Valli VE: Techniques in veterinary cytopathology. Semin Vet Med Surg (Small Anim) **3**:85–93, 1988.
55. Valli VE: T-cell and NK-cell neoplasms. *In:* Veterinary Comparative Pathology, pp. 309–311, Blackwell, Ames, IA, 2007.
56. Valli VE, Jacobs RM, Parodi AL, Vernau W, Moore PF: Histological Classification of Hematopoietic Tumors of Domestic Animals. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, 2002.
57. Valli VE, Vernau W, de Lorimier LP, Graham PS, Moore PF: Canine indolent nodular lymphoma. Vet Pathol **43**:241–56, 2006.
58. Willemze R, Kerl H, Sterry W, Berti E, Cerroni L, Chimenti S, Diaz-Perez JL, Geerts ML, Goos M, Knobler R, Ralfkiaer E, Santucci M, Smith N, Wechsler J, van Vloten WA, Meijer CJ: EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. Blood **90**:354–371, 1997.